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Gymnopus fuscotramus (*Agaricales*), a new species from southern China

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Abstract — A new species, *Gymnopus fuscotramus,* is described from China. It is characterized by brown-incarnate colors in pileus and lamellae, sulcate pileus, free and distant lamellae, floccose-squamulose, mostly black stipe, well-developed black rhizomorphs, repent and diverticulate pileipellis hyphae, abundant clamp connections, diverticulate to coralloid cheilocystidia, moderately thick-walled caulocystidia with obtuse apex, dextrinoid hyphae in cortex of stipe, and gray-brown pileal and hymenophoral trama. Color images of basidiomata and microscopic elements accompany the description. *Gymnopus fuscotramus* is compared with similar species and its systematic position is also inferred using the ITS rDNA sequence data.

Key words — *Basidiomycota,* biodiversity, *Omphalotaceae,* taxonomy

Introduction

During field research on marasmioid fungi in southern China (Guangxi Zhuang Autonomous Region), the third author collected an interesting fungus which is described here as a new species of the genus *Gymnopus* (Pers.) Roussel. According to morphological characters, it belongs to section *Androsacei* (Kühner) Antonín & Noordel., which is characterized by small and marasmioid basidiomata, thin, insititious and mostly dark colored stipe, usually well-developed black rhizomorphs, dextrinoid trama of stipe and non-hymeniform pileipellis (Antonín & Noordeloos 2010). Members of this section were traditionally placed in the genus *Marasmius* (Fries 1838). Kühner (1933) described section *Androsacei* under the same genus. On the basis of

its non-hymeniform pileipellis, Antonín (1987) excluded members of the section *Androsacei* from *Marasmius,* and described a new genus *Setulipes*. Molecular phylogenetic studies published by Moncalvo et al. (2002), Mata et al. (2004) and Wilson & Desjardin (2005) showed that the type species of the genus *Setulipes*, *S. androsaceus* (L.) Antonín, belongs to the gymnopoid clade. Therefore, Noordeloos & Antonín (2008) transferred section *Androsacei* to the genus *Gymnopus*.

Materials & methods

The description of *Gymnopus fuscotramus* is based on one collection consisting of seven basidiomata, which were photographed in the field. Color codes in the macroscopic description (given in brackets) are cited according to Kornerup & Wanscher (1981). Microscopic features were observed with a light microscope (brightfield and phase contrast – PhC) under magnification up to 1500× and photographed with a digital camera. Description and images of microscopic characters were made from rehydrated specimens mounted in 2.5% potassium hydroxide (KOH) solution. Amyloidity and dextrinoidity were tested in Melzer's reagent (Erb & Matheis 1983). Basidiospore measurements were calculated from mounts of lamellae and based on calibrated digital images. A total number of 50 randomly selected basidiospores from two mature basidiomata were measured. Spore measurements (length, width) are given as: (min.) stat. min. – av. – stat. max. (max), where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic average minus two times standard deviation), "av." = arithmetic average, "stat. max." = statistical maximum (arithmetic average plus two times standard deviation), "max." = maximum (highest measured value). Standard deviation (SD) of spore length and width is also given. The length/width ratio of spores is given as the "Q" value (min. – av. – max.). The holotype is deposited in the Herbarium of Guangdong Institute of Microbiology (GDGM), while an isotype is deposited in the Croatian National Fungarium (CNF). Comparison of *Gymnopus fuscotramus* with similar taxa is based on revision of type specimens of *Marasmius nigroimplicatus* Corner (E 206719) and *M. subrigidichorda* Corner (E 206861), as well as descriptions in the following literature: Petch 1948, Singer 1976, 1989, Pegler 1986, Desjardin 1987a,b, Corner 1996, and Antonín 2007.

Genomic DNA was isolated from dried material with E.Z.N.A. forensic kit (Omega bio-tek) according to manufacturer's protocol for isolation of DNA from hair, nails and feathers. The primers ITS1F and ITS4 (White et al. 1990) were used for amplification and sequencing of ITS region, containing the ITS1, 5.8S and ITS2 regions of rDNA. PCR amplifications were performed in a total volume of 25 µl. The initial denaturation step at 95 °C for 85 s, was followed with 35 cycles of 94 °C for 35 s, 55 °C for 45 s, and 72 °C for 60 s. PCR products were subcloned into pGEM-T vector (Promega) according to the manufacturer's instructions. Three positive clones were sequenced using the pUC or T7 vector primers with the ABI BigDye Ready Reaction Kit on an ABI 3100 automated sequencer. Sequencing reads were assembled using Lasergene processing software (DNASTAR Inc., Madison, USA) and checked manually for sequencing errors. Sequence, submitted to GenBank with accession number JF303730, was compared

Figs 1–2. *Gymnopus fuscotramus* (holotype). 1. Basidiomata in situ. 2. Pileipellis (PhC). Bars: 1 = 10 mm; 2 = 10 μm.

by homology searches with known sequences using BLAST (Benson et al. 2003). ITS sequences from 25 species taken from NCBI were used for further analysis. An alignment of the sequences was performed using Clustal X (Thompson et al. 1994). Ambiguously aligned regions were determined and excluded from further analyses using the online version of the program Gblocks 0.91b, under less stringent parameters (Castresana 2000). Final alignment was 620 bp long (available upon request). Jmodeltest (Posada 2008, Guindon & Gascuel 2003) was used to select the best-fit model of nucleotide substitution. Phylogenetic analyses were performed using Bayesian MCMC, maximum parsimony (MP) and Neighbor-joining (NJ) methods. Four species of *Marasmius* were selected as outgroup taxa for rooting purposes. Bayesian inference of the phylogeny using Metropolis coupled Markov chain Monte Carlo analyses (Geyer 1991) was performed using MrBayes, version v. 3.1.2. (Ronquist & Huelsenbeck 2003), under the Hasegawa-Kishino-Yano + gamma (HKY+G) model, which incorporates different rates for transitions and transversions and rate variation across sites. MCMC sampling was performed as implemented in MrBayes with the default settings (two runs of four chains each) for 10,000 generations, with the first 10% discarded as burn-in. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities (PPs) of the groups. Branch lengths were computed as the mean values over the trees sampled after burn-in. MP and NJ analyses were performed in Mega version 4 (Tamura et al. 2007). Relative robustness of individual branches was estimated by bootstrapping (BS), using 1000 replicates.

Taxonomy

Gymnopus fuscotramus Mešić, Tkalčec & Chun Y. Deng, **sp. nov.** Figs 1–7

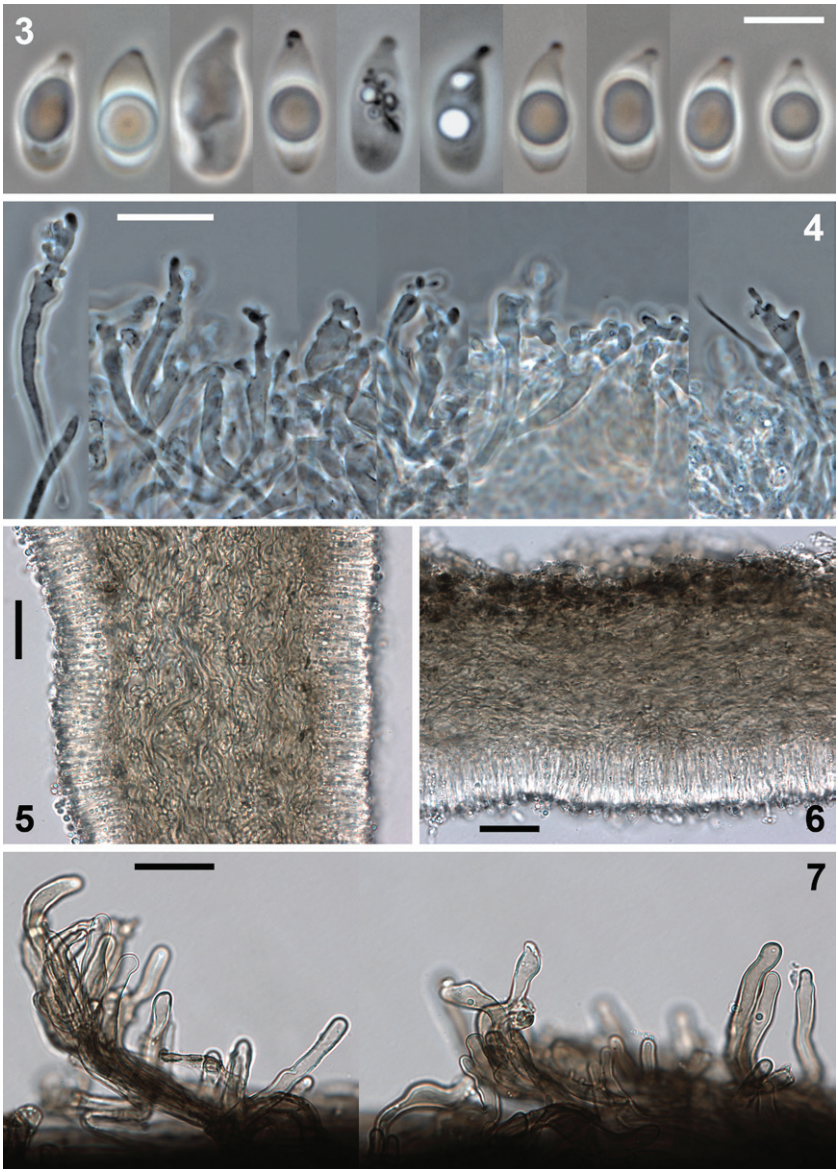
MycoBank MB 519324

Pileus 12–21 mm latus, campanulatus, sulcatus, brunneolo-incarnatus usque incarnato-brunneus. Lamellae liberae, distantes, brunneolo-incarnatae. Stipes 15–30 × 1–1.5 mm, floccoso-squamulosus, apice aurantio- usque rubro-brunneus, humilius nigro-brunneus usque niger, insiticius. Sporae (6.7–)6.8–8.2–9.6(–9.8) × (3.0–)3.0–3.7–4.4(–4.8) µm, oblongae, subcylindricae, amygdaliformes vel lacrimiformes, hyalinae. Cheilocystidia 20–45(–60) × 5–15(–25) µm, subcylindrica usque irregulariter clavata et diverticulata vel coralliformia. Pleurocystidia absentia. Caulocystidia 5–100 × 4–12 µm, plerumque cylindrica, subcylindrica vel anguste clavata, apice obtusa, crasse tunicata (0.5–2 µm). Pileipellis cutis, hyphis diverticulatis. Trama pilealis et hymenophoralis fusca. Fibulae abundantes. Hyphae in corticali stipite dextrinoideae.

Etymology: Named for the gray-brown color of its pileal and hymenophoral trama.

Holotype: CHINA, GUANGXI: Maoershan Nature Reserve, 72 km N of Guilin, 25°54'32"N, 110°27'30"E, alt. 1500 m, 29 May 2009, leg. C.Y. Deng, GDGM 26313. Isotype: CNF 1/6044.

Pileus 12–21 mm broad, campanulate, sometimes with applanate or slightly depressed center, sulcate almost to the center, weakly translucently striate when moist, hygrophanous, brownish incarnate to incarnate-brown when moist (from 10B4 to 11D5), pale incarnate on drying, with darker, mostly dark red-brown to blackish-brown center, surface dull, dry. Lamellae free (without



Figs 3–7. *Gymnopus fuscotramus* (holotype). 3. Spores (PhC). 4. Cheilocystidia (PhC). 5. Hymenophoral trama. 6. Pileal trama. 7. Caulocystidia. Bars: 3 = 5 μm; 4 = 20 μm; 5–7 = 30 μm.

a collarium or pseudocollarium), distant (L = ca. 12, l = 1), ≤2 mm broad, ventricose, reaching the margin of the pileus or almost so, sometimes slightly intervenose, brownish incarnate, with entire, concolorous edge. Stipe 15–30 × 1–1.5 mm, subcylindrical, orange- to red-brown in the upper part, downward brown-black to black, entirely pale brown floccose-squamulose, dry, hollow, insititious, arising directly from substrate (not from rhizomorphs). Context grayish-brown and very thin in pileus, whitish in stipe medulla, concolorous with surface in stipe cortex. Smell and taste not recorded. Rhizomorphs abundant, filiform, unbranched, ≤135 mm long and ≤0.5 mm thick, glabrous, black, with black to black brown inner context, hollow, apex with white globule or tapering and concolorous.

Spores [50/2/1] (6.7–)6.8–*8.2*–9.6(–9.8) × (3.0–)3.0–*3.7*–4.4(–4.8) µm, SD = 0.71 × 0.34, Q = 1.73–*2.23*–2.70, oblong to subcylindrical, in side view often amygdaliform or even lacrymoid, smooth, hyaline, thin-walled, non-amyloid, non-dextrinoid. Basidia 23–36 × 4.5–6 µm, narrowly clavate, (2)4-spored, thin-walled, hyaline, clamped. Basidioles narrowly clavate, cylindrical or fusoid. Lamellar edge sterile, composed of repent, diverticulate, hyaline to gray-brown, 1.5–8 µm broad hyphae, with clusters of cheilocystidia. Cheilocystidia 20–60 × 5–15(–25) µm, subcylindrical to irregularly clavate with irregular finger- to knob-like projections or coralloid, thin- to moderately thick-walled (≤0.8 µm thick), hyaline to pale gray-brown. Pleurocystidia absent. Hymenophoral trama irregular, composed of 1–6(–8) µm broad, thin- to moderately thick-walled (≤1 µm thick), subhyaline to pale gray-brown (gray-brown in mass) hyphae, pigment intracellular. Pileipellis a subregular cutis composed of 1.5–10 µm broad, mostly thin-walled, less frequently moderately thick-walled (≤0.8 µm thick), subhyaline to brown, mostly diverticulate hyphae, with occasional coralloid elements, dark brown pigment often coarsely encrusted. Pileal trama composed of pale gray-brown (gray-brown in mass), non-gelatinized, thin- to moderately thick-walled (≤0.8 µm thick), 1–7 µm broad hyphae, pigment intracellular. Stipitipellis a cutis of parallel, 1.8–7 µm broad, brown hyphae with intracellular, sometimes also encrusted pigment. Caulocystidia very abundant, 5–100 × 4–12 µm, mostly cylindrical, subcylindrical or narrowly clavate, with obtuse apex, sometimes diverticulate, moderately thick-walled to thick-walled [walls 0.5–2(–2.5) µm thick], sometimes with one septa, often in groups, subhyaline to brown, with intracellular, sometimes also encrusted pigment. Stipe trama composed of parallel, thin- to thick-walled (≤1.2 µm thick), 2–10 µm broad hyphae, hyaline in stipe medulla. Clamp connections present and abundant in all tissues. Chemical reactions: all parts of basidioma non-amyloid and non-dextrinoid except hyphae in cortical layer of stipe and caulocystidia, which are dextrinoid (red-brown in Melzer's reagent).

Habitat — In small clusters on a twig, in humid subtropical forest of *Fagus longipetiolata* and *Castanopsis lamontii*.

Distribution — Known only from the type locality in China.

Discussion

Gymnopus fuscotramus is characterized by a brown-incarnate colors in pileus and lamellae, sulcate pileus, free and distant lamellae, floccose-squamulose, mostly black stipe, well-developed black rhizomorphs, growing only on woody substrate (not arising from rhizomorphs), non-hymeniform pileipellis (cutis with diverticulate hyphae), abundant clamp connections, diverticulate to coralloid cheilocystidia, moderately thick-walled caulocystidia with obtuse apex, dextrinoid hyphae in cortex of stipe, and gray-brown pileal and hymenophoral trama.

Among other species in the section *Androsacei* with clamp connections, black rhizomorphs, non-glabrous stipe, and colored pileus and lamellae (not white or cream), *Marasmius bactrosporus* Singer, *M. campinaranae* Singer, and *Setulipes brevistipitatus* Antonín have (among others) much smaller basidiomata and more elongated spores. *M. nigroimplicatus* has thinner (≤0.2 mm) and minutely pubescent rhizomorphs, adnate lamellae sometimes attached to a pseudocollarium, more elongated spores, and caulocystidia with thicker walls (1.8–5 µm). *M. rigidichorda* Petch has basidiomata arising also from the rhizomorphs, adnate lamellae, white context, hyaline hymenophoral trama, and caulocystidia with pointed apex. *M. subrigidichorda* has basidiomata arising also from the rhizomorphs, lamellae attached to a pseudocollarium, longer and wider rhizomorphs (≤410 × 0.9 mm), hyaline hymenophoral trama, and caulocystidia with thicker walls (2–5 µm). *M. thiersii* Desjardin has crowded, adnate to adnexed lamellae, rare or absent rhizomorphs, and lacks cheilocystidia. *Setulipes rhizomorphicola* Antonín has much shorter (≤3 mm) and eccentric stipe arising from rhizomorphs, adnate lamellae, and longer spores.

According to morphological characters, *Gymnopus fuscotramus* belongs to section *Androsacei*. However, our phylogenetic analysis using only ITS rDNA sequences do not place our taxon on the same clade with the two sequenced species from that section, *G. androsaceus* (L.) J.L. Mata & R.H. Petersen and *G. quercophilus* (Pouzar) Antonín & Noordel. However, *G. fuscotramus* is placed close to *G. peronatus* (Bolton) Gray (sect. *Vestipedes*). To further clarify phylogenetic relationships between *G. fuscotramus* and other *Gymnopus* species (especially from sect. *Androsacei*), more species (reliably identified) and more DNA sequences should be included in analyses. Our gymnopoid clade also includes *Marasmiellus* and *Rhodocollybia* species, which is in accordance with results of Moncalvo et al. (2002), Mata et al. (2004) and Wilson

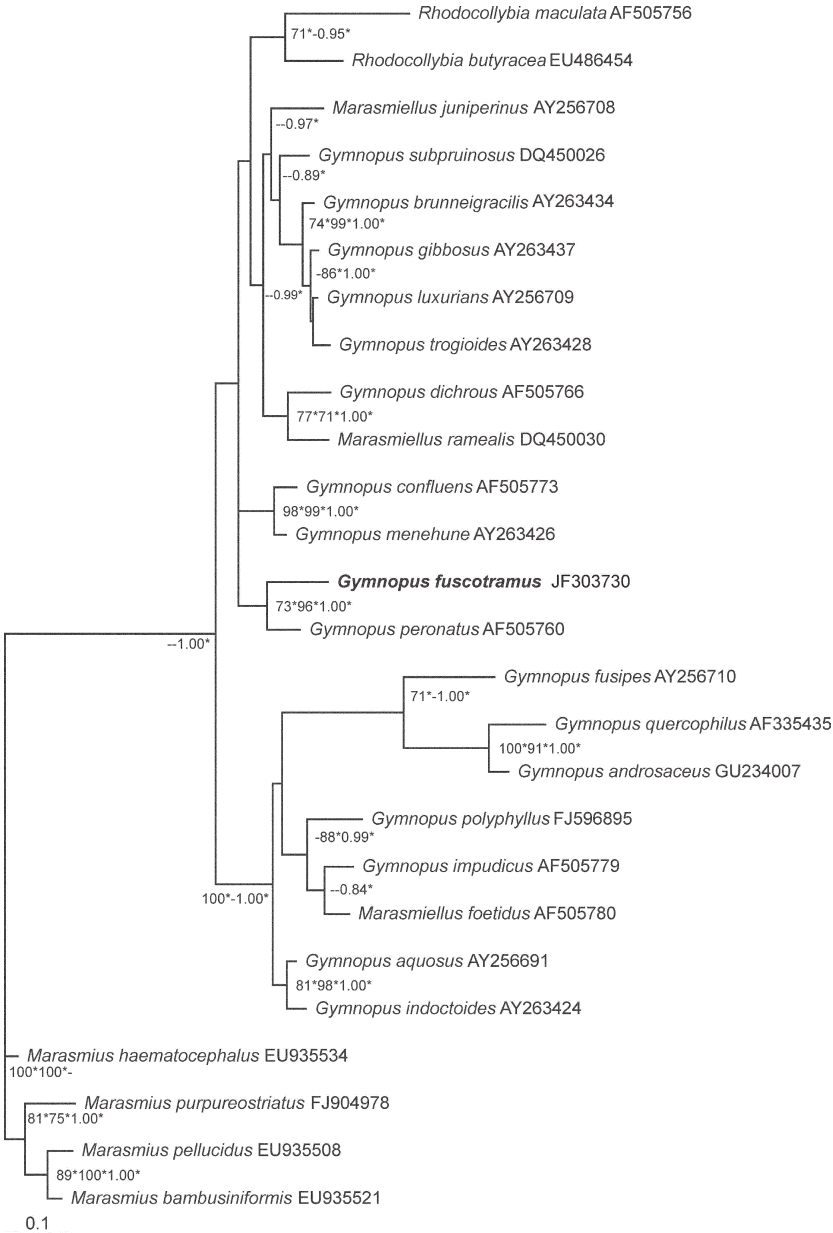


Fig. 8. Phylogenetic tree based on the complete ITS rDNA region (ITS1, 5.8S rDNA, and ITS2), showing mean branch lengths of a 50 % majority-rule consensus tree from a Bayesian MCMC analysis. NJ, MP bootstrap values (>70%), and Bayesian PP values are given above nodes.

& Desjardin (2005). All these results indicate that *Gymnopus* and *Marasmiellus* (as conceived recently) are polyphyletic and that new taxonomic concepts inferred from DNA sequences should be proposed. However, since past phylogenetic analysis included only a small number of species from these two genera, further taxonomic solutions should be based on more extensive phylogenetic research with more species and DNA sequences included.

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